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REVIEW

Stereochemical Control in Lipase Reaction

Kaoru NAKAMURA*, Masamichi KINOSHITA*
and Atsuyoshi OHNO*

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Enzymatic reactions are being increasingly employed for a variety of asymmetric syntheses. This review provides methods for controlling stereochemistry in lipase-catalyzed reaction, such as (1) water content, (2) medium effect, (3) modification of substrates, (4) temperature, and (5) additives.

KEY WORDS: Lipase/ Log *P*/ *E*-value/ Transesterification

1. INTRODUCTION

Among organic chemists, it has become generally recognized that several enzymes, in particular, lipases (triacylglycerol acyl-hydrolases EC 3.1.1.3) can exert catalytic activity in organic solvents.[1-3] There are many advantages in conducting enzymatic reactions in organic media: (1) efficient catalysis may be achieved for substrates poorly soluble in water; (2) substrates unstable in water may be subjected to the reaction; (3) hydrolytic enzymes can catalyze esterification reactions; (4) enhancement of thermostability; and (5) products and enzymes can easily be recovered from reaction system. Actually, by employing lipases in organic solvents, a great number of racemic alcohols are resolved to the corresponding chiral alcohols with high enantiomeric excess.[1-3] When a high enantioselectivity is required, screening of enzymes is an efficient method. However, another method, control of reaction conditions, is also effective and is useful for organic chemists. In fact, many parameters influence the selectivity of enzymatic reactions in organic media. Hence, chemists need to determine the optimum conditions for the highest selectivity by altering these parameters individually. The purpose of this review article is to prospect factors that may affect stereoselectivity of lipase-catalyzed reactions.

2. STEREOCHEMICAL CONTROL

2.1 Water content

Enzymes inherently act in aqueous environment, so they need a considerable amount of water to keep their activity as catalysts in organic media. This water, called essential water, protects enzymes from organic solvent and it makes enzymes sufficiently flexible to be induced into the active conformation by interaction with a substrate.[4] That is to say, essential water

* 中村 薫, 木下雅道, 大野惇吉 : Bioorganic Chemistry I, Institute for Chemical Research, Kyoto University, Uji, Kyoto 611, Japan.

plays a role of lubricant for enzymes. Recently the role of added water on the enzyme, subtilisin Carlsberg, suspended in organic media has been reported.[5] Although dried enzyme has little activity, water added to the suspension increases the polarity at the active site sharply to make the enzyme more active. Interestingly, added water increases the rate of acylation of enzyme and decreases K_m of acylating reagent in the transesterification of *N*-Ac-L-Phe ethyl ester with 1-propanol catalyzed by subtilisin BPN'.[6] This phenomenon is also related to the flexibility of the enzyme. In the transesterification of 1-octanol with vinyl butyrate catalyzed by *Pseudomonas cepacia* lipase (PcL), K_m of 1-octanol increases with increasing water activity (a_w) but V_{max} assigns an optimum value at intermediate a_w and decreases at both low and high a_w conditions.[7] According to the authors, the decrease in V_{max} at high a_w is attributed to physical aggregation of suspended enzyme powder or modification of the interface between phases.[7] Increase in K_m is due to the decrease in distribution of the alcohol from bulk solvent to the enzyme or inhibition caused by butyric acid which is produced by hydrolysis of vinyl butyrate.

Since water can affect both the conformation and activity of enzymes, chemists have the possibility of enhancing the catalytic activity and stereoselectivity of enzymatic reactions by controlling water content in reaction systems. Of course, water content in the reaction medium is closely related with the nature of organic solvent, because a part of water is dissolved in the organic phase while the remainder locates in a more polar phase around the enzyme. The effect of organic solvents will be discussed later.

There are several methods in regulating water content in reaction mixture: (1) addition of water,[8-16] (2) hydration or dehydration of enzymes,[17-19] (3) addition of moleclar sieves,[20] and (4) addition of salt hydrates.[21,22] Addition of water means direct addition of water to the reaction system, and hydration of enzyme means another technique to hydrate an enzyme directly. Both methods are utilized for the purpose of increasing water content in enzyme powder. On the other hand, in order to decrease the water content in enzyme powder or medium, we usually dehydrate an enzyme under reduced pressure or add molecular sieves to the reaction medium. In almost all cases, enzymes have maxima in their activity against the content of water. So, it is necessary to find the maximum activity or selectivity by changing water content. These methods can not be applied to esterification of alcohol with a carboxylic acid, because water is produced as the reaction proceeds and the content of water in reaction mixture can not be maintained to be a constant. Addition of salt hydrate is a unique way to regulate the water content.[21,22] When a hydrated salt (*e.g.* $Na_2SO_4 \cdot 10H_2O$) and its corresponding anhydrous salt (*e.g.* Na_2SO_4) are present together in a reaction mixture, activity of water in the system can be maintained to be a constant, because the pair of salts releases or takes up water as required. These salts behave like a buffer for water activity. The water activity in the reaction mixture is theoretically affected by the kind of salt hydrate pair only. We have only to seek the best pair of salts proper for the experimental conditions. These are summarized in Table 1.

As shown in Table 1, hydrophobic solvents are employed when the effect of water content on enzymatic reactions is investigated, since water is efficiently distributed to enzyme particles in such solvents. Water affects not only the activity but also enantioselectivity of the lipase in the esterification of 2-bromopropionic acid with 1-butanol catalyzed by *Candida rugosa* (formerly *cylindracea*) lipase (CrL) in organic media.[13] Water affects the activity only remaining the enantioselectivity unchanged in transesterification of sulcatol with viny acetate or 2, 2, 2-

Table 1. Effect of Water Content on Lipase-Catalyzed Reactions.

Origin of lipase	Substrate	Solvent	Ref.
addition of water			
Pseudomonas cepacia	vinyl acetate methyl 3-hydroxy octanoate	dodecane	8
	vinyl acetate sulcatol	toluene	9
Pseudomonas sp.	vinyl acetate sulcatol	dodecane	9
Pseudomonas fluorscens	15-hydroxypentadecanoic acid	benzene	10
Pseudomonas aeruginosa	vinyl acetate (a)	toluene	11
Candida rugosa	2-chloropropionic acid 1-butanol	hexane	12
	2-bromopropionic acid 1-butanol	hexane	13
	tributyrin 1-octanol	hexane etc.	14
	tributyrin heptanol	2-pentanone	15
Mucor sp.	tributyrin heptanol	2-pentanone	15
porcine pancreas	tributyrin heptanol	2-pentanone	15
	tributyrin 1-heptanol	tributyrin	16
hydration or dehydration of enzymes			
Pseudomonas cepacia	vinyl butyrate 1-octanol	toluene	17
Mucor miehei	decanoic acid 1-dodecanol	hexane etc.	18
porcine pancreas	tributyrin sulcatol	ether	19
addition of molecular sieves			
Pseudomonas cepacia	trifluoroethyl butyrate sulcatol	toluene etc.	20
porcine pancreas	trifluoroethyl butyrate sulcatol	toluene etc.	20
addition of salt hydrates			
Candida rugosa	butanoic acid butanol	hexane	21
	2-methyl octanoic acid 1-dodecanol	cyclohexane	22

(a): (±)-(3aα, 4α, 6aα)-1, 3-dibenzyl-3a, 6, 6a-tetrahydro-4-hydroxy-1*H*-thieno[3, 4-*d*]imidazol-2(3*H*)one.

trifluoroethyl butyrate catalyzed by PcL.[9] However, in the transesterfication of methyl 3-hydroxyoctanoate with vinyl acetate catalyzed by PcL, increase in water content leads to decrease in both activity and enantioselectivity.[8] Thus optimized content of water depends considerably on the other reaction conditions such as enzyme, substrate, and solvent.

2.2 Medium effect

Several enzymes are able to retain their catalytic activity in nonaqueous media. In addition, enzymes in organic solvents also have abnormal thermostability.[16] These characteristics of enzymes in organic solutions are explained on the basis of increased rigidity of proteins in nonaqueous media. Practically, electron paramagnetic resonance (EPR) reveals that the flexibility of protein decreases dramatically with decrease in solvent dielectric constant.[23] Furthermore, molecular dynamics simulations of a protein in chloroform show that substitution of the surrounding medium from water to chloroform strongly influences the geometries of the amino acid side chains.[24] Therefore, it is not surprising even though an enzyme exerts a high catalytic activity in polar media because of its flexibility. However, polar solvents are generally hydrophilic and interact so strongly with water molecules that essential water surrounding an enzyme is stripped by the solvents. Since essential water is the most important factor for an enzyme to keep the activity in nonaqueous medium, a lipase is more active in hydrophobic (nonpolar) solvents than in hydrophilic (polar) solvents.[25] Unlike mold and yeast lipases, pancreatic lipase has no relationship between the catalytic activity and $\log P$, an index representing hydrophobicity of solvents,[25] probably because pancreatic lipase is topologically affected by several other factors such as cofactor, colipase, and bile salts *in vivo*.[26]

It is difficult to interpret the relationship between enzymic stereoselectivities and solvents systematically, because solvents have many kinds of characters such as polarity, hydrophobicity, electron pair donor (or acceptor) property and structure.[27] Polarity and hydrophobicity of solvents seem to be powerful candidates that affect stereoselectivities of enzymes. In transesterifications catalyzed by proteases, enantioselectivities are correlated linearly with dipole moment[28] and hydrophobicity[29] of solvents. These correlations are accounted for by rigidity of enzyme and thermodynamic stability of enzyme-substrate complex.

Solvent effect on stereoselectivity of lipase-catalyzed reaction has also been investigated unsuccessfully. Stereoselectivities in transesterifications catalyzed by PcL are summarized in Table 2.

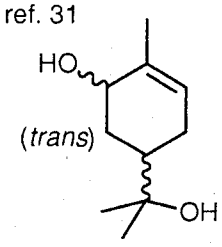
Although enough data for explanation are not obtained yet, several features can be found. It is obvious that enantioselectivity does not simply depend on the hydrophobicity of solvents. Ketones, alkylarenes and alkanes are more efficient solvents for the enantioselectivity of lipase than (cyclo) aliphatic ethers and aliphatic alcohols. However, this hypothesis is not applied to the substrates having polar substituent such as hydroxyl and ester group. It has been reported that acetone-water solvent system is more efficient for the enantioselectivity than water in the hydrolysis of alkan-2-yl acetates catalyzed by PcL.[34] Acetone interacts with PcL specifically.

In esterifications[35,36] and hydrolyses[37] catalyzed by CrL, enantioselectivity varies drastically by changing the solvent. In these reactions, interestingly, carboxylic part, instead of alcoholic part, has chiral center. This enzyme has frequently been utilized to resolve racemic carboxylic acids[38] and the structure has been determined by X-ray diffraction method.[39,40] The conformation of acyl-binding pocket is considered to be affected largely by the nature of

Table 2. Effect of Solvents on the Enantioselectivity of Transesterification catalyzed by *Pseudomonas cepacia* lipase.

Solvent	log *P*	*E*-Value[30]			
		ref. 31	ref. 20	ref. 32	ref. 33
Dioxane	−1.1	178	23		4
Dichloromethane	−0.5			6.7	5
Acetone	−0.2	142	40		17
Vinyl acetate	0.3	89		1.7	10
Tetrahydrofuran	0.5	69	27		
3-Pentamome	0.8	212	47		5
2-Methyl-2-butanol	1.4	518	20		
Diisopropyl ether	1.9			2.8	
3-Methyl-3-pentanol	2.0		16		
Benzene	2.0		32		
Toluene	2.5		34		5
Dibutyl ether	2.9		22		
Cyclohexane	3.1		13	2.2	
Hexane	3.5			4.5	16
Dodecane	6.6		21		16

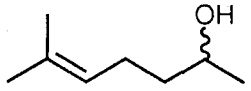
ref. 31



and

Vinyl acetate

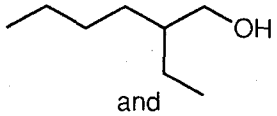
ref. 20



and

Trifluoroethyl butyrate

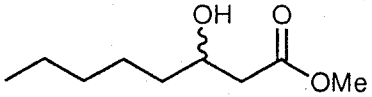
ref. 32



and

Vinyl acetate

ref. 33



and

Vinyl acetate

solvent. It has been recognized that the flap of CrL to cover the active site is opened easily by changing the environmental conditions.[39,40] Hence, the solvent is expected to change the structure of substrate-binding region to open or close the flap of lipase.

Recently, it has been reported that catalytic activity of lipase is affected by chiral solvent such as (*R*)- or (*S*)-carvone.[41] Structure of solvent molecule also affects the stereoselectivity of transesterification between 4-methylcyclohexanol and vinyl acetate catalyzed by *Pseudomonas* sp. lipase (AK) (see Figure 1).[42]

Solvent effect on prochiral selectivity[43,44] and regioselectivity[45] of lipase has been reported. In the lipase AH (from *Pseudomonas* sp.)-catalyzed hydrolysis of prochiral bis (alcoxymethyl)-1, 4-

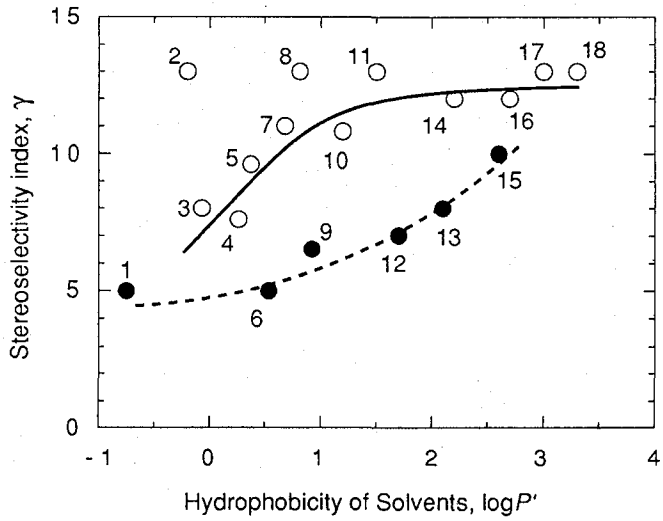


Fig. 1. Correlation between hydrophobicity of solvent and stereoselectivity of *Pseudomonas* sp. lipase in the transesterification of *cis*- and *trans*-methylcyclohexanols with vinyl acetate. Solvent: (1) dioxane, (2) acetonitrile, (3) acetone, (4) propionitrile, (5) 2-butanone, (6) tetrahydrofuran, (7) ethyl acetate, (8) diethyl ether, (9) 2-methyltetrahydrofuran, (10) tert-butyl methyl ether, (11) diisopropyl ether, (12) benzene, (13) toluene, (14) dibutyl ether, (15) cyclohexane, (16) hexane, (17) heptane, (18) octane. (●): cyclic solvents, (○): acyclic solvents [ref. 42].

dihydro-3, 5-pyridine dicarboxylates,[43] the lipase attacks the pro-(*R*)-ester selectively in water saturated diisopropyl ether. In contrast, the same lipase attacks the pro-(*S*)-ester in water saturated cyclohexane. Since the other lipase (PcL) attacks pro-(*S*)-product preferentially in both solvents, the authors have concluded that this solvent effect arises from specific interactions between the solvent and lipase AH. The prochiral selectivity correlates inversely with log *P* of organic solvent in the hydrolysis of prochiral 2-(1-naphthoylamino) trimethylenedibutyrate catalyzed by lipase AK.[44] From these observation, we conclude that these lipases interact specifically with organic solvents. The regioselectivity roughly correlates with log *P* of the solvent in the transesterification of 1, 4-dibutyryloxy-2-octylbenzene with butanol catalyzed by PcL.[45] This solvent effect is accounted for by the partition of octyl moiety in the substrate between the solvent and binding cleft of lipase.

First of all, we should focus the interest on a particular enzyme in order to elucidate the factors by which solvent influences enzymatic specificity. Secondly, we should classify organic solvents into groups such as alkanes, ethers, ketones and alkyl arenes, and then elucidate the effect of these solvents on enzymatic specificities in each group. Investigation of solvent effect will resolve mechanism of enzymatic reactions in detail.

2.3 Modification of substrates

In order to resolve chiral alcohols by using lipase-catalyzed esterification, choice of acyl donor is a quite important factor for controlling the stereochemistry. Vinyl acetate is the most frequently used acyl donor in transesterfication.[46-48] Enol esters, represented by vinyl acetate,

make acyl-transfer reactions irreversible because the leaving vinyl alcohol is converted into acetaldehyde. However, produced acetaldehyde inhibits enzymatic activity and enantioselectivity through formation of Schiff's base with amino residues of lysine as seen in the transesterification of endo-bicyclo [2.2.1] hept-5-en-2-ol with vinyl acetate catalyzed by CrL.[49] Actually this lipase has some lysines such as Lys 75 and Lys 85 which exist in the flap region.[40] The flap in lipoprotein lipase has α-helices which are amphipathic.[50] It has been reported that reduction of the amphipathlicity abolishes the ability of lipase to hydrolyze emulsified triglycerides.[50] The presence of a flap may be one of the important factors for the activity and stereoselectivity of lipase in organic solvents. CrL has an amphipathlic α-helix in the flap region,[40] in which Lys 75 is contained. The formation of Schiff's base with Lys 75 may break the amphipathlicity and the lipase may lose not only the enzymatic activity but also the enantioselectivity.

The fatty acid moiety of the vinyl esters affects the reaction rate and enantioselectivity of acylation in lipase AK-catalyzed acylation of methyl mandelate in diisopropyl ether.[51] The enantioselectivity of acylation changes from *S* to *R* by switching the vinyl ester from acetate to chloroacetate (see Figure 2).

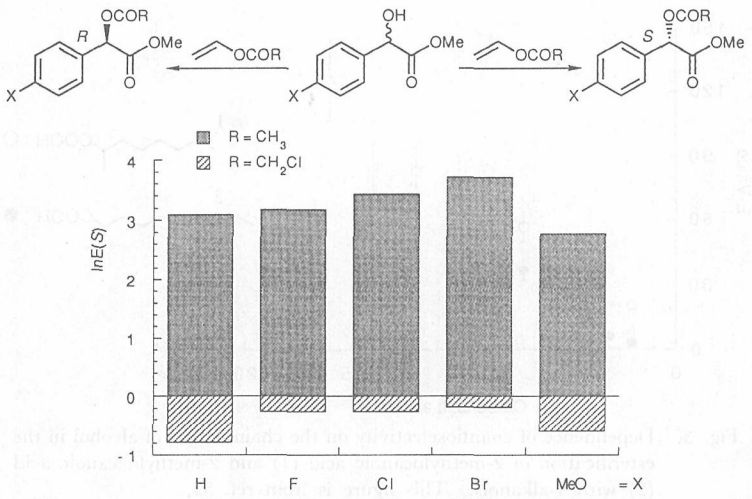


Fig. 2. Inversion of enantioselectivity by switching acyl donor from vinyl acetate to vinyl chloroacetate, in the transesterification catalyzed by *Pseudomonas* sp. lipase. *E*-values[30] were calculated from data reported in ref. 51.

Although *R*-selectivity is not high compared to *S*-selectivity, this is the first example which shows that the acyl donor can invert the enantioselectivity of the lipase-catalyzed transesterification. Since bulkiness of the fatty acid moiety of the vinyl ester is not related with the inversion of enantioselectivity,[51] an electron withdrawing property of chlorine atom may influence the enantioselectivity of acylation dramatically.

Vinyl acetate and vinyl hexanoate afford excellent reactivity and enantioselectivity in the acylation of (±)-(3aα, 4α, 6aα)-1, 3-dibenzyl-3a, 4, 6, 6a-tetrahydro-4-hydroxy-1*H*-thieno [3, 4 *d*] imidazol-1-2(3*H*)-one in toluene catalyzed by lipase from *Pseudomonas aeruginosa*.[11] The

introduction of a phenyl group or a chlorine atom in acylating agents causes a detrimental effect on reactivity and enantioselectivity.

The enantioselectivity of acylation increases *ca.* 4-fold by changing the acyl donor from tributyrin to 2, 2, 2-trifluroethyl laurate in porcine pancreatic lipase (PPL)-catalyzed acylation of sulcatol in diethyl ether.[19] This increase of enantioselectivity is mainly due to two factors; the carbon chain-length of acyl donor and the regulation of reverse reaction.

2, 2, 2-Trichloroethyl methoxyacetate is more effective to the enzymic activity than vinyl acetate in the acylation of 1-hexanol catalyzed by CrL.[52] Thus, methoxyacetate exerts a high specificity for CrL. Unfortunately, the effect of this acyl donor on enantioselectivity has not been investigated.

Furthermore, it was reported that the chain length of acyl acceptor affects the enantioselectivity of esterification catalyzed by CrL.[53] In the esterification of 2-methyloctanoic acid, the enantioselectivity of lipase increases monotonously with the increase in the carbon chain-length in primary alkanols. On the other hand, in the esterification of 2-methyldecanoic acid, the increase in enantioselectivity is observed when the chain length is less than 6 and the selectivity decreases at longer substrates (see Figure 3).

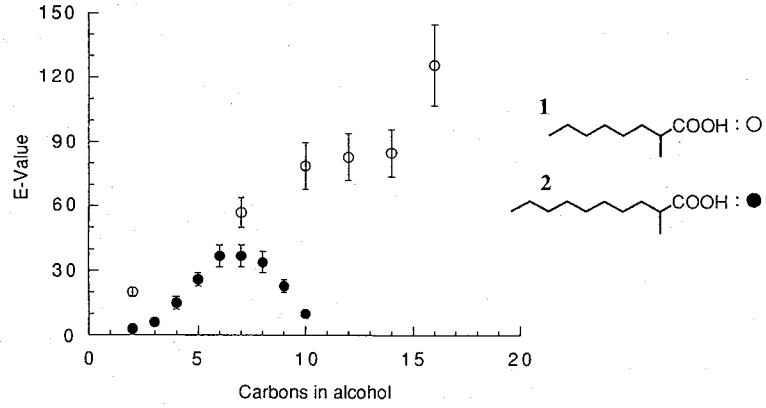


Fig. 3. Dependence of enantioselectivity on the chain length of alcohol in the esterification of 2-methylocanoic acid (1) and 2-methyldecanoic acid (2) with 1-alkanols. This figure is from ref. 53.

It is worth noting that, even though the structures of **1** and **2** are not so different (see Figure 3), enantioselectivity differs very much.

2.4. Temperature

It is well known that reaction rate increases with increasing temperature in enzymatic reactions up to denaturation of the enzyme as is seen in chemical reactions.[54] It has already been reported that the specificity of enzyme is inversely proportional to temperature, because the flexibility of enzyme increases with the increase in temperature.[55,56] In the transesterification of allylic alcohols with cyclohexyl acetate catalyzed by PcL, enantioselectivity of the reaction decreases with the increase in reaction temperature.[57] Similar tendency is seen in both the transesterification of sulcatol catalyzed by PPL and of 3-bromo-5-hydroxymethyl isoxazoline

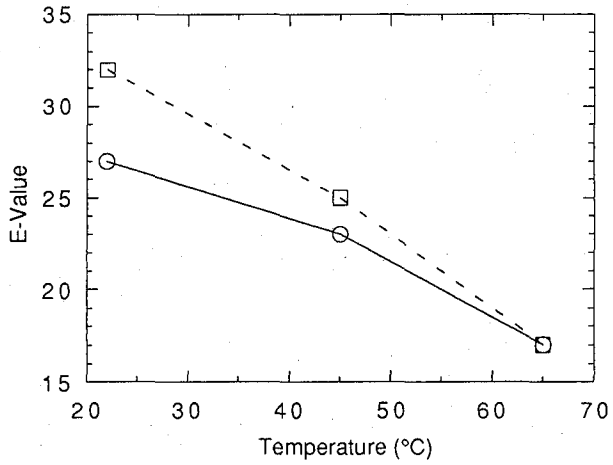


Fig. 4. Effect of temperature on enantioselectivity of porcine pancreatic lipase with sulcatol (○), and of *Pseudomonas cepacia* lipase with 3-bromo-5-hydroxymethyl isoxazoline (□). Data are cited from ref. 20.

catalyzed by PcL in the presence of 2, 2, 2-trifluoroethyl butyrate (see Figure 4).[20]

On the other hand, the enantioselectivity of CrL decreases with the increase of temperature in the reaction with 2, 2-dimethyl-1, 3-dioxolane-4-methanol, whereas the lipase does not show clear dependency of enantioselectivity on temperature in the case of 3, 7-dimethyl-6-octenal as the substrate.[58]

2.5 Additives

Additives except water also influence enzymatic reactions. Actually, it has recently been reported that a salt dramatically enhances activity of subtilisin Carlsberg suspended in an organic solvent.[59] Importantly, the increase of enzymatic activity is seen only when the enzyme forms a complex with a salt, which suggests that intricate interaction between the enzyme and salt is necessary in order to exert the effect: no enhancement of enzymic activity is observed when the same concentration of salt is added to the reaction mixture containing salt-free enzyme powder, because the salt is insoluble to the organic solvent and cannot be distributed into enzyme powder in organic media.

Thus, first of all, before concluding the effectiveness of the additive, we must confirm the distribution of additive into the enzyme in a reaction system.

Secondly, the additive should be defined as a reagent which can exert an effect by its trace amount. Addition of 1 M of 1-naphthol decreases prochiral selectivity about 10-fold in the hydrolysis of 2-(1-naphththoylamino) trimethylelnedibutyrate to the corresponding monobutyrate in acetonitrile catalyzed by lipase AK.[44] Addition of D-sorbitol is effective to enantioselectivity in the hydrolysis of prochiral *cis*-cyclohex-4-ene-1, 2-bis (methylacetate) with porcine liver esterase in phosphate buffer solution. However, surprisingly, the amount of D-sorbitol required is 7.3 g (40 mmol) per 1 mmol of the substrate in 30 ml of buffer solution.[60] In both cases, materials added as the additives are much enough to change the properties of media such as polarity, and these cannot be claimed as the additives.

Amines are effective additives to enzymatic reactions. Dextromethorphan is found to be an

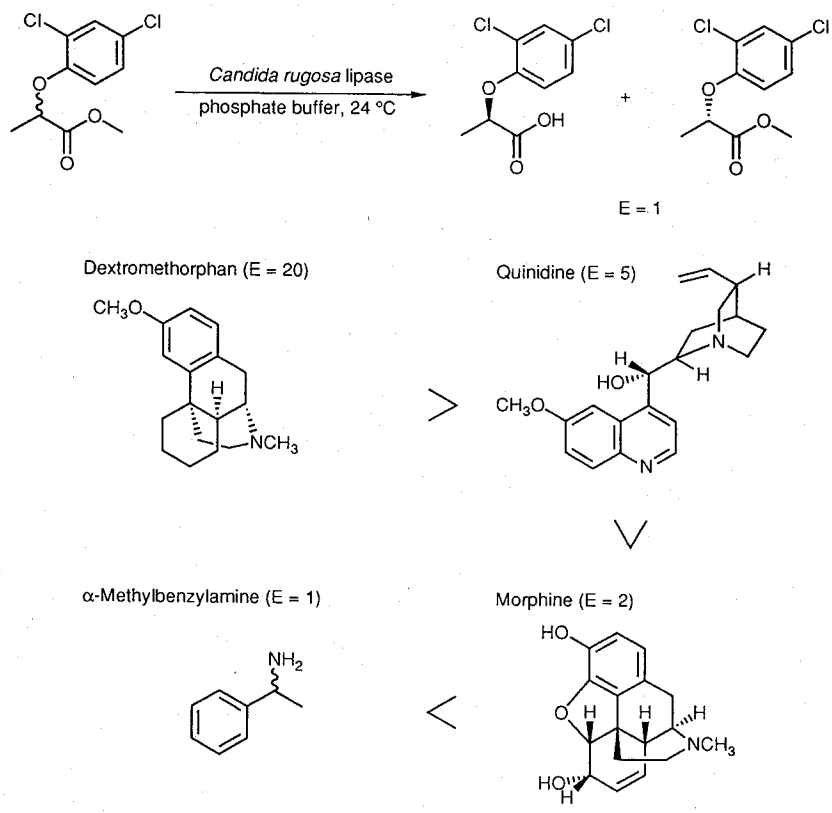


Fig. 5. Effect of chiral amines on the lipase-catalyzed enantioselective hydrolysis of methyl 2-(2, 4-dichlorophenoxy)-propionate. A sigh of inequality means the order of effectiveness to the enantioselectivity.

effective enantioselective inhibitor (see Figure 5) for the hydrolysis of *rac*-methyl 2-(2, 4-dichlorophenoxy) propionate (DCPP) catalyzed by CrL in phosphate buffer.[61] Mechanism of enantioselective inhibition is interpreted as partial noncompetitive inhibition for (*R*)-DCPP and pure noncompetitive inhibition for (*S*)-DCPP. Moderate improvement of enantioselectivity is noted with quinidine and morphine, whereas *α*-methylbenzylamine is not effective at all.

Pyrrolidine gives a minor improvement to enantioselectivity in the hydrolysis of glycidyl butyrate catalyzed by PPL in buffer-sarurated organic solvents.[62] The result may mean that amines are more effective to enzymatic specificty in water than in organic media. Furthermore, amino alcohols have been known to increase the enantioselectivity about 2-fold in the PcL-catalyzed hydrolysis of 3-acetoxybutyronitrile in phosphate buffer.[63] Bases such as 2, 6-lutidine (2, 6-dimethylpyridine) and potassium hydrogen carbonate enhance the enantioselectivity about 10-fold and 12-fold, respectively, in the CrL-catalyzed transesterification of 7, 7-dimethoxy 1, 4, 5, 6-tetrachlorobicyclo [2.2.1] hept-5-en-2-ols with acetic anhydride.[64] The effect of base stems from the removal of carboxylic acid formed as a co-product.

Molecular sieve is one of the efficient additives to regulate water content in a reaction mixture. However, it is not a reagent to modify the enzyme directly, but an adsorbent of specific molecules such as water and methanol.

3. CONCLUSION

Factors, influencing enzymatic reactions, are dependent upon each other. Water content in an enzyme powder is closely related to the solvent property. The structures and electronic properties of acyl donor and acceptor affect the stability and reactivity of acyl enzyme, so these are also related with each other through complexes with an enzyme. The quantity of additives may vary the property of reaction medium. Temperature influences the property of a solvent such as viscosity. However, since enzymatic reactions are usually conducted in a small range of temperature, temperature seems to be independent of other factors and not to affect the specificity of enzyme greatly. Hence, it is very difficult to determine optimum conditions for high stereoselectivity.

Although there still remains many subjects to be solved before the enzymes are used in organic chemistry, the approach will undoubtedly make enzymes more powerful tools in the region of organic syntheses.

REFERENCES

(1) C.-S. Chen and C.J. Sih, *Angew. Chem., Int. Ed. Engl.*, **28**, 695 (1989).
(2) A.M. Klibanov, *Acc. Chem. Res.*, **23**, 114 (1990).
(3) M.N. Gupta, *Eur. J. Biochem.*, **203**, 25 (1992).
(4) A. Zaks and A.M. Klibanov, *J. Biol. Chem.*, **263**, 3194 (1988).
(5) R. Affleck, Z.-F. Xu, V. Suzawa, K. Focht, D.S. Clark and J.S. Dordick, *Proc. Natl. Acad. Sci. USA*, **89**, 1100 (1992).
(6) P.P. Wangikar, T.P. Graycar, D.A. Estell, D.S. Clark and J.S. Dordick, *J. Am. Chem. Soc.*, **115**, 12231 (1993).
(7) R. Bovara, G. Carrea, G. Ottolina and S. Riva, *Biotechnol. Lett.*, **15**, 937 (1993).
(8) U. Bornscheuer, A. Herar, L. Kreye, V. Wendel, A. Capewell, H.H. Meyer, T. Scheper and F.N. Kolisis, *Tetrahedron: Asymmetry*, **4**, 1007 (1993).
(9) R. Bovara, G. Carrea, G. Ottolina and S. Riva, *Biotechnol. Lett.*, **15**, 169 (1993).
(10) T. Yamane, Y. Kojima, T. Ichiryu, M. Nagata and S. Shimizu, *Biotechnol. Bioeng.*, **34**, 838 (1989).
(11) S. Tokuyama, T. Yamano, I. Aoki, K. Takanohashi and K. Nakahama, *Chem. Lett.*, **1993**, 741.
(12) J. Bodnár, L. Gubicza and L.-P. Szabó, *J. Mol. Catal.*, **61**, 353 (1990).
(13) H. Kitaguchi, I. Itoh and M. Ono, *Chem. Lett.*, **1990**, 1203.
(14) H. Hirata and K. Higuchi, *Yukagaku*, **36**, 643 (1987).
(15) A. Zaks and A.M. Klibanov, *Proc. Natl. Acad. Sci. USA*, **82**, 3192 (1985).
(16) A. Zaks and A.M. Klibanov, *Science*, **224**, 1249 (1984).
(17) R. Bovora, G. Carrea, G. Ottolina and S. Riva, *Biotechnol. Lett.*, **15**, 937 (1993).
(18) R. Valivety, P.J. Halling and A.R. Macrae, *Biochim. Biophys. Acta*, **1118**, 218 (1992)
(19) T.M. Stokes and A.C. Oehlschlager, *Tetrahedron Lett.*, **28**, 2091 (1987).
(20) F. Secundo, S. Riva and G. Carrea, *Tetrahedron: Asymmetry*, **3**, 267 (1992).
(21) L. Kvittingen, B. Sjursnes, T. Anthonsen and P. Halling, *Tetrahedron*, **48**, 2793 (1992).
(22) H.-E. Högberg, H. Edlund, P. Berglund and E. Hedenström, *Tetrahedron: Asymmetry*, **4**, 2123 (1993).
(23) R. Affleck, C.A. Haynes and D.S. Clark, *Proc. Natl. Acad. Sci. USA*, **89**, 5167 (1992).
(24) D.S. Hartsough and K.M. Merz, Jr., *J. Am. Chem. Soc.*, **114**, 10113 (1992).
(25) C. Laane, S. Boeren, K. Vos and C. Veeger, *Biotechnol. Bioeng.*, **30**, 81 (1987).
(26) H. van Tilbeurgh, M.-P. Egloff, C. Martinez, N. Rugani, R. Verger and C. Cambillau, *Nature*, **362**, 814 (1993).
(27) C. Reichardt, "Solvents and Solvent Effects in Organic Chemistry", 2nd ed., VCH, Weinheim, Germany (1988).
(28) P.A. Fitzpatrick and A.M. Klibanov, *J. Am. Chem. Soc.*, **113**, 3166 (1991).
(29) S. Tawaki and A.M. Klibanov, *J. Am. Chem. Soc.*, **114**, 1882 (1992).
(30) C.-S. Chen, Y. Fujimoto, G. Girdaukas and C.J. Sih, *J. Am. Chem. Soc.*, **104**, 7294 (1982).

(31) R. Bovara, G. Carrea, L. Ferrara and S. Riva, *Tetrahedron : Asymmetry,* **2**, 931 (1991).
(32) S. Barth and F. Effenberger, *Tetrahedron : Asymmetry,* **4**, 823 (1993).
(33) U. Bornscheuer, A. Herar, L. Kreye, V. Wendel, A. Capewell, H.H. Meyer, T. Scheper and F.N. Kolisis, *Tetrahedron : Asymmetry,* **4**, 1007 (1993).
(34) Y. Naoshima, M. Kamezawa, H. Tachibana, Y. Munakata, T. Fujita, K. Kihara and T. Raku, *J. Chem. Soc., Perkin Trans. 1,* **1993**, 557.
(35) S.-H. Wu, F.-Y. Chu and K.-T. Wang, *Bioorg. Med. Chem. Lett.,* **1**, 339 (1991).
(36) S. Ueji, R. Fujino, N. Okubo, T. Miyazawa, M. Kitadani and A. Muromatsu, *Biotechnol. Lett.,* **14**, 163 (1992).
(37) M.J. García, R. Brieva, F. Rebolledo and V. Gotor, *Biotechnol. Lett.,* **13**, 867 (1991).
(38) G. Kirchner, M.P. Scollar and A.M. Klibanov, *J. Am. Chem. Soc.,* **107**, 7072 (1985).
(39) P. Grochulski, Y. Li, J.D. Schlag, F. Bouthillier, P. Smith, D. Harrison, B. Rubin and M. Cygler, *J. Biol. Chem.,* **268**, 12843 (1993).
(40) P. Grochulski, Y. Li, J.D. Schlag and M. Cygler, *Protein Science,* **3**, 82 (1994).
(41) G. Ottolina, F. Gianinetti, S. Riva and G. Carrea, *J. Chem. Soc., Chem. Commun.,* **1994**, 535.
(42) K. Nakamura, M. Kinoshita and A. Ohno, *Tetrahedron,* **50**, 4681 (1994).
(43) Y. Hirose, K. Kariya, I. Sasaki, Y. Kurono, H. Ebiike and K. Achiwa, *Tetrahedron Lett.,* **33**, 7157 (1992).
(44) F. Terradas, M. Teston-Henry, P.A. Fitzpatrick and A.M. Klibanov, *J. Am. Chem. Soc.,* **115**, 390 (1993).
(45) E. Rubio, A. Fernandez-Mayorales and A.M. Klibanov, *J. Am. Chem. Soc.,* **113**, 695 (1991).
(46) Y.-F. Wang and C.-H. Wong, *J. Org. Chem.,* **53**, 3127 (1988).
(47) Y.-F. Wang, S. T. Chen, K.K.-C. Liu and C.-H. Wong, *Tetrahedron Lett.,* **30**, 1917 (1989).
(48) K.A. Babiak, J.S. Ng, J.H. Dygos, C.L. Wayker, Y.-F. Wang and C.-H. Wong, *J. Org. Chem.,* **55**, 3377 (1990).
(49) B. Berger and K. Faber, *J. Chem. Soc., Chem. Commun.,* **1991**, 1198.
(50) K.A. Dugi, H.L. Dichek, G.D. Talley, H.B. Brewer, Jr. and S. Santamarina- Fojo, *J. Biol. Chem.,* **267**, 25086 (1992).
(51) T. Miyazawa, S. Kurita, S. Ueji, T. Yamada and S. Kuwata, *J. Chem. Soc., Perkin Trans. 1,* **1992**, 2253.
(52) V.F. Hogan, D. O'Hagan and J. Sanvoisin, *Indian J. Chem.,* **31B**, 883 (1992).
(53) P. Berglund, M. Holmquist, E. Hedenström, K. Hult and H.-E. Högberg, *Tetrahedron : Asymmetry,* **4**, 1869 (1993).
(54) I.H. Segel, "Enzyme Kinetics," John Wiley & Sons, New York (1975).
(55) V.T. Pham, R.S. Phillips and L.G. Ljungdahl, *J. Am. Chem. Soc.,* **111**, 1935 (1989).
(56) L.K.P. Lam, R.A.H.F. Hui and J.B. Jones, *J. Org. Chem.,* **51**, 2047 (1986).
(57) U. Bornscheuer, S. Schapöhler, T. Scheper and K. Schügerl, *Tetrahedron : Asymmetry,* **2**, 1011 (1991).
(58) V.S. Parmar, A.K. Prasad, P.K. Singh and S. Gupta, *Tetrahedron : Asymmetry,* **3**, 1395 (1992).
(59) Y.L. Khmelnitsky, S.H. Welch, D.S. Clark and J.S. Dordick, *J. Am. Chem. Soc.,* **116**, 2647 (1994).
(60) Y. Nagao, T. Tohjo, T. Kaneuchi, Y. Yukimoto and M. Kume, *Chem. Lett.,* **1992**, 1817.
(61) Z.-W. Guo and C.J. Sih, *J. Am. Chem. Soc.,* **111**, 6836 (1989).
(62) J.L.L. Rakels, A.J.J. Straathof and J.J. Heijnen, *Tetrahedron : Asymmetry,* **5**, 93 (1994).
(63) T. Itoh, E. Ohira, Y. Takagi, S. Nishiyama and K. Nakamura, *Bull. Chem. Soc. Jpn.,* **64**, 624 (1991).
(64) B. Berger, C.G. Rabiller, K. Königsberger, K. Faber and H. Griengl, *Tetrahedron : Asymmetry,* **1**, 541 (1990).